Week 7 Notebook

July 13, 2015 - July 17, 2015

July 13, 2015

Kayla/Julie

- Miniprepped 31C liquid cultures
    - planned a 15 µL test digest, but accidentally added PstI to minipreps 1 and 2 instead of the reaction tubes
    - Miniprep 3 digest was run on a 1.0% agarose gel for 1 hour at 90V
        - no band was present, possibly due to lack of DNA in the digest reaction tube
- Prepared a new biofilm assay plate with EMG2:Kλ, ZeAFP in EMG2:Kλ, and RiAFP in EMG2:Kλ in LB and M9 minimal media
    - LB Wells
        - A1-D1 and A10-D10=EMG2:Kλ
        - A2-D2 and A11-D11=ZeAFP in EMG2:Kλ
        - A3-D3 and A12-D12=RiAFP in EMG2:Kλ
    - M9 Wells
        - E1-H1 and E10-H10=EMG2:Kλ
        - E2-H2 and E11-H11=ZeAFP in EMG2:Kλ
        - E3-H3 and E12-H12=RiAFP in EMG2:Kλ
- Grew new 5mL liquid cultures for 31C

Chloe/Charlotte

A new freeze assay was attempted, based on Wang et al., 2010, “An improved 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay for evaluating the viability of *Escherichia coli* cells,” with the following procedure:

- Take OD600 of liquid culture
- Dilute to OD = 0.1 in LB/glycerol (7.9 mL glycerol in 1 L LB)
- Repeated 4 times: 200 ul of diluted culture + 40 ul MTS solution, mixed well
- Incubate at 37°C for 20 minutes with caps open
- Centrifuge at 1 minute at 13.2 g to pellet, then put cultures on ice
- Place 100 ul of supernatant from each in a 96-well flat-bottomed plate, plus 100 ul blank (LB/glycerol) in two wells
- Read on plate reader at 450 nm (actual wavelength is 490, but plate reader has only a few presets)
- Place 0.5 ml of initial undiluted culture in -20 and -80 freezers in slow-freeze container and repeat the previous steps for those cultures 24 hours later

All of the synthesized AFP sequences in stock were used for in a PCR using the new primers and the 3' suffix primer to amplify the AFP sequences without a start codon. The resulting PCR products were purified and eluted in 50 uL of water instead of the standard 30 uL.

Cultures of RiAFP, ZeAFP, J23119 were prepared and left to incubate in the shaker overnight for use in a run of the new freeze survival assay. Cultures of RFP and Chloe Clone GFP were prepared as well for painting.

Dave/Eddie

- Digested all 16 PCR amplified AFPs with X and P for two hours, then heat killed the enzyme and column purified.
- MoAFP sequencing looks good! We now have three AFPs ligated with J23119 (:
- Picked colony 2 for both 15-23C and 15-43C for growth overnight.
- Ligated the other 13 AFPs with 15-17C overnight (2 ul vector, 5 ul insert, 20 ul total)

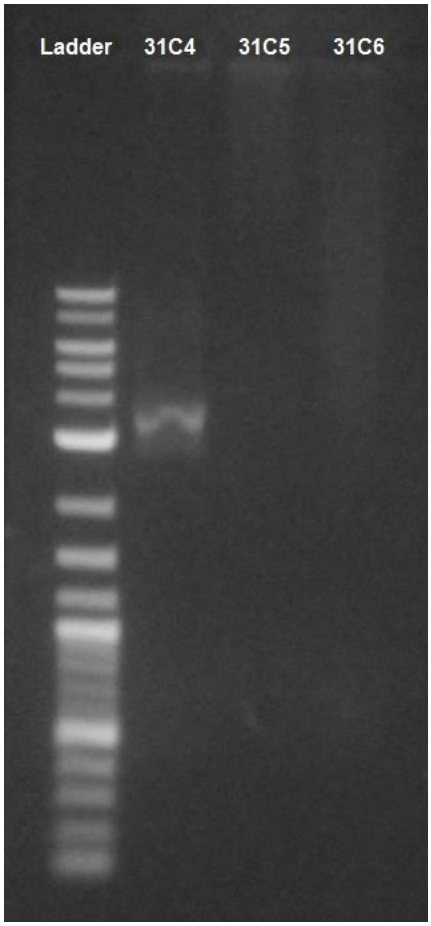
July 14, 2015

Kayla/Julie

- Miniprepped liquid cultures for 31C
    - concentrations

Miniprep	DNA Concentration (ng/µL)
31C4	543
31C5	786
31C6	1068

    - test digested with XbaI and PstI
        - 12 uL reaction
    - ran test digest on a gel for 1 hour at 90V
        - no bands present in lanes for minipreps 5 and 6
            - possible that sample was not loaded into the gel correctly
            - possible that DNA concentrations were lower than expected
        - sent miniprep 4 for sequencing



- Prepared 100 mL liquid cultures of GFP, BclA-YFP, and RFP expressing strains for women in science day camp

Chloe/Charlotte
Restriction digests of all the purified PCR products from yesterday using X and P were prepared. Digests of 27C and 28C with S and P were prepared. The digests were incubated at 37°C for 2(?) hours.

A ligation of 14 of the AFPs (Ep, Cf, Ch, Br, Dc, Hh, Mo, Ri, Lp, **list the rest**) into 28C was performed, using:

- 5 ul insert
- 2 ul backbone
- 10 ul water
- 1 ul ligase
- 2 ul ligase buffer

The cultures of RiAFP, ZeAFP, and J23119 (15-17C, 17-19C, and 15-21C) were back-diluted and grown up again overnight, since we ran out of time to perform the MTS freeze assay.

Dave/Eddie

- Made glycerol stocks and miniprepped 15-23C and 15-43C, then sent samples away for sequencing.
- Transformed the ligations from yesterday and the miniprep of MoAFP.
- Painted pictures for the girls camp the following day.

July 15, 2015

Kayla/Julie

- Women in science day camp
- Stained biofilm plate
    - no robust biofilm formation in positive control or in the strains expressing antifreeze proteins
        - inconclusive experiment

Chloe/Charlotte

An additional restriction digest of 27C (40 ul) and 28C (20 ul) with S and P was performed to prepare for more ligations. They were incubated at 37°C for 2.5 hours.

The first part of the MTS freeze assay was performed with 15-17C, 15-19C, and 15-21C: 500 ul cultures were placed in the -20 and -80 freezers (at 4:15 pm), and the MTS procedure was performed with the pre-freeze cultures.

Dave/Eddie

- We got many colonies from the transformations, we will conduct colony PCR to try and identify properly transformed colonies.
- Made more 3' primer dilution using the bbsuffix 3' stock.
- Conducted colony PCR on 48 colonies and a positive control (ZeAFP) and a negative control from the control plate.
- Colony PCR
    - 1st well in each lane is ladder, last two wells of each are a positive control and negative control respectively.
    - wells as follows: strain (number of colonies picked)

1) 38C (6)	3) 42C (2)	5) 20C (3)	7) 32C (3)	9) 34C (6)	11) 36C (6)	13) 39C (1)
2) 41C (3)	4) 44C (3)	6) 22C (6)	8) 33C (3)	10) 35C (1)	12) 37C (3)	14) 40C (2)

July 16, 2015

Kayla/Julie

- Transformed 15-17C (negative control), 15-36C, and 15-43C into EMG2:Kλ

Chloe/Charlotte

The rest of the AFPs were transformed into 28C, and all 16 AFPs were transformed into 27C.

Dave/Eddie

- Pick colonies to grow in liquid cultures to miniprep
    - Mp#2
    - Dc#2
    - Ng#1
    - Hh#1,2
    - Ga#3
    - Br#5
    - Ch#1
    - Ma#2
    - Ep#1
    - Lp#1
    - Mo#6 (already sequence confirmed, this is done only for glycerol stock, which was forgotten)
    - Ap#2
    - Cf#1
    - IA#1
- Added seq data for TiAFP
- We have TiAFP!!
- Digested PpAFP using X and P in a 50ul reaction
- Ligated TmAFP again and transformed. The column purification was skipped as it was forgotten
- we need more wash buffer, PE may be an acceptable substitute. Recipe found here: http://2013.igem.org/wiki/images/8/85/Homemade_Buffer_Compositions.pdf

July 17, 2015

Kayla/Julie

- Checked transformation plates
    - MoAFP in EMG2:Kλ had large colonies
    - TiAFP in EMG2:Kλ and J23117 in DH5α had a lawn of small colonies
    - BBa_I20270 in DH5α had no colonies
- Transformations were repeated
    - ZeAFP and RiAFP were re-transformed into EMG2:Kλ
    - 26C was transformed into EMG2:Kλ

- Plates were streaked with 29C5, 30C1, and 31C4 glycerol stocks to obtain biological replicates for the interlab study

Chloe/Charlotte

The transformations of the 14 AFPs into 28C were run through colony PCR, using the 3' and 5' iGEM primers and Dave's protocol.

Approximate lengths of AFP sequences + GFP from 28C (in kb)																	
Ze	Mo	Ep	IAFGP	Ma	Ap	Cf	Lp	Tm	Ch	Br	Ng	Ti	Hh	Cs	Mp	Dc	Pp
1.3	1.3	1.4	1.5	1.5	1.5	1.5	1.6	1.6	1.7	1.7	1.9	1.9	1.9	2	2.2	2.2	2.7

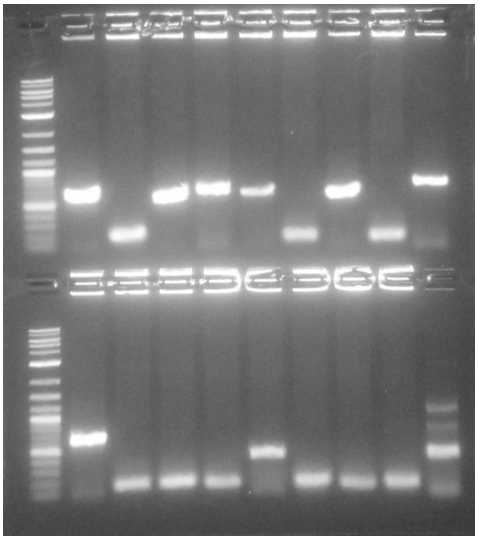
Approximate lengths of AFP sequences + BclA from 27C (in kb)																	
Ze	Mo	Ep	IAFGP	Ma	Ap	Cf	Lp	Tm	Ch	Br	Ng	Ti	Hh	Cs	Mp	Dc	Pp
.43	.49	.56	.63	.62	.68	.68	.72	.71	.8	.85	1.09	1.09	1.03	1.12	1.33	1.35	1.78

Once the colony PCR was complete, the products were run between three gels, pictured below.

Gel 1				Gel 2				Gel 3		Ctrl	
1*	2	3*	4*	5*	6	7*	8	9*	10	11	12
IAFGP + BclA	IAFGP + BclA	IAFGP + BclA	Lp + BclA	Lp + BclA	Lp + BclA	Cf + BclA	Cf + BclA	Cf + BclA	Ch + BclA	Ch + BclA	Ch + BclA
0.63 kb			.72 kb			0.68 kb			0.8 kb		
13*	14	15	16	17	18	19*	20	21	22*	23*	24*
Ze + BclA	Ze + BclA	Ze + BclA	Ga + BclA	Ga + BclA	Ga + BclA	Hh + BclA	Hh + BclA	Hh + BclA	Ri + BclA	Ri + BclA	Ri + BclA
0.43 kb			0.85 kb			1.03 kb			0.69 kb		
25	26*	27	28*	29	30*	31*	32	33	34	35	361
Mp + BclA	Mp + BclA	Mp + BclA	Dc + BclA	Dc + BclA	Dc + BclA	Ti + BclA	Ti + BclA	Ti + BclA	Ap + BclA	Ap + BclA	Ap + BclA
1.33 kb			1.35			1.09			0.68		
37*	38	39	40	41	42	43	44				
Ep + BclA	Ep + BclA	Ep + BclA	Br + BclA	Br + BclA	Br + BclA	GFP (neg. ctrl)	Ze (pos. ctrl)				

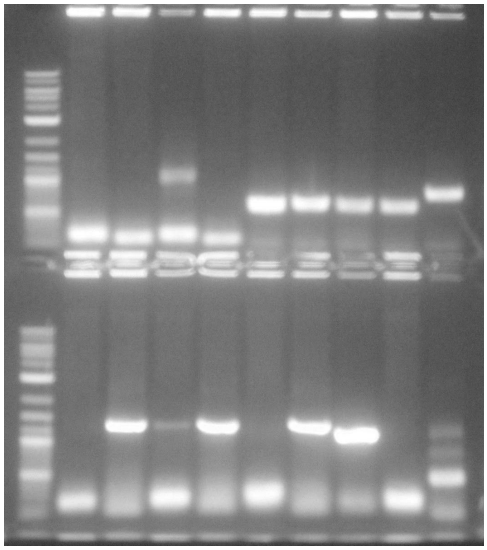
0.56 kb	0.85 kb						

Gel 1:



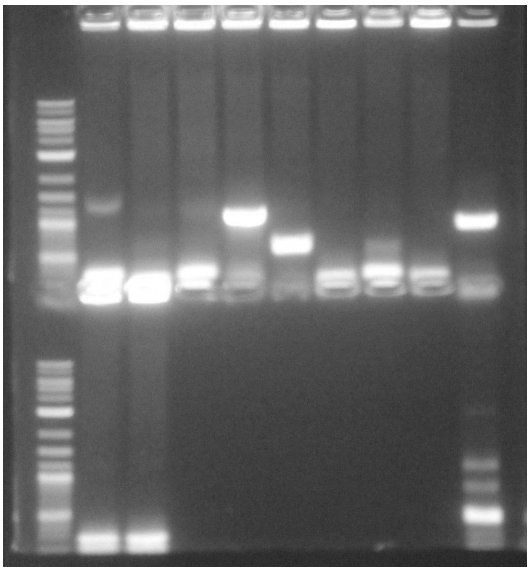
Lanes 1A and 1B: ladder
Lanes 2A-9A: samples 1-8
Lanes 2B-9B: samples 9-16
Lane 10A: sample 43 (negative control)
Lane 10B: sample 44 (positive control)

Gel 2:



Lanes 1A and 1B: ladder
Lanes 2A-9A: samples 17-24
Lanes 2B-9B: samples 25-32
Lane 10A: sample 43 (negative control)
Lane 10B: sample 44 (positive control)

Gel 3:



Lanes 1A and 1B: ladder
Lanes 2A-9A: samples 33-40
Lanes 2B-3B: samples 41-42
Lane 10A: sample 43 (negative control)
Lane 10B: sample 44 (positive control)

Schematic of 64-well plate (last two columns removed); OD at 450 nm, key below										
	1	2	3	4	5	6	7	8	9	10
A	0.075	0.08	0.465	0.464	0.46	0.486	0.563	0.575	0.568	0.558
B	0.077	0.078	0.379	0.366	0.384	0.395	0.329	0.337	0.325	0.32
C	0.079	0.078	0.543	0.567	0.574	0.622	0.373	0.377	0.4	0.4
D	0.096	0.102	0.509	0.463	0.444	0.448	0.479	0.474	0.502	0.474
E	0.098	0.098	0.433	0.416	0.431	0.45				
F	0.333		0.725	0.6	0.62	0.54	0.422	0.428	0.372	0.469
G			0.631	0.666	0.659	0.721	0.564	0.55	0.44	0.578
H			0.453	0.911	0.614	0.53	0.486	0.468	0.456	0.747

A 1-2, B 1-2, D 1-2, E 1-2: LB + glycerol
F 1: LBG + MTS

A 3-6: 34C before freeze
B 3-6: 34C after -20 freeze

B 7-10: 34C after -80 freeze
A 7-10: 35C before freeze
C 3-6: 35C after -20 freeze
C 7-10 35C after -80 freeze
D 3-6: 17C before freeze
F 3-6: 17C after -20 freeze
F 7-10: 17C after -80 freeze
D 7-10: 19C before freeze
G 3-6: 19C after -20 freeze
G 7-10: 19C after -80 freeze
E 3-6: 21C before freeze
H 3-6: 21C after -20 freeze
H 7-10: 21C after -80 freeze

LBG + MTS (blank): 0.333

34C MTS Freeze Assay Results (OD_{600})				
Pre-freeze	0.465	0.464	0.46	0.486
After -20 freeze	0.379	0.366	0.384	0.395
After -80 freeze	0.329	0.337	0.325	0.32

35C MTS Freeze Assay Results (OD_{600})				
Pre-freeze	0.563	0.575	0.568	0.558
After -20 freeze	0.543	0.567	0.574	0.622
After -80 freeze	0.373	0.377	0.4	0.4

17C MTS Freeze Assay Results (OD_{600})				
Pre-freeze	0.509	0.463	0.444	0.448
After -20 freeze	0.725	0.6	0.62	0.54
After -80 freeze	0.422	0.428	0.372	0.469

19C MTS Freeze Assay Results (OD_{600})				
Pre-freeze	0.479	0.474	0.502	0.474
After -20 freeze	0.631	0.666	0.659	0.721
After -80 freeze	0.564	0.55	0.44	0.578

21C MTS Freeze Assay Results (OD_{600})				
Pre-freeze	0.433	0.416	0.431	0.45
After -20 freeze	0.453	0.911	0.614	0.53
After -80 freeze	0.486	0.468	0.456	0.747

Dave/Eddie

- We got colonies for TmAFP, we will pick seven and test using colony PCR
    - the negative control from the 15th was used, and ZeAFP was the positive control
- We glycerol stocked and minprepped the picked colonies from yesterday and sent them away for sequencing.
    - Due to a mishap during miniprepping, 5 tubes labeled with one AFP got mixed up, so the actual contents are unknown. We still sent them away and will check each against all five possibilities.
    - All AFPs less than 1kb were sent in only for forward reading, those over 1kb also got reverse reading. Only Mp and Dc were read in both directions.
- A test digest was conducted of the picked 15-36C colony.

July 19, 2015

Kayla/Julie

- Prepared 5 mL liquid cultures of EMG2:Kλ, 26C, MoAFP, RiAFP, and TiAFP